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Patents ADP number *(if you know it)*	00798652002
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DUPLICATE

Glycosylphosphatidylinositol Specific Phospholipase D Proteins and Uses Thereof

Field of the Invention

The present invention relates to glycosylphosphatidylinositol specific phospholipase D (GPI-PLD) proteins and uses of these proteins, in particular in the treatment and diagnosis of conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock.

Background of the Invention

Studies have shown that a number of cell surface proteins are attached to the cell membrane by covalent linkage to a glycosylphosphatidylinositol (GPI) anchor. It has been shown that the enzyme GPI-PLD cleaves the photodiester bond linking glycosylphosphatidylinositol to phosphatidic acid, thereby releasing anchored proteins.

GPI-PLD enzymes are abundantly present in human and bovine serum (5-10µg/ml in human serum). US Patent No: 5,418,147 (Huang et al) describes the purification of GPI-PLD from bovine liver, and the subsequent cloning of three GPI-PLD enzymes from bovine liver, human liver and human pancreas cDNA libraries. This patent reports the full length cDNA and amino acid sequences of the GPI-PLDs from human and bovine liver, and the partial cDNA and amino acid sequences of the human pancreatic form of the enzyme. Subsequently, the full length sequence of the pancreatic form of GPI-PLD was reported in Tsang et al (1992), and this enzyme has been found in cDNA libraries from breast, eye, spleen and tonsil. The three forms of the enzymes are highly homologous with the predicted mature protein sequences of bovine liver GPI-PLD sharing 82% sequence identity with the human liver enzyme and 81% sequence identity with the human pancreatic enzyme. The amino acid sequences of human liver and pancreatic forms of GPI-PLD were deposited at GenBank under accession numbers L11701 and L11702 and consist of 841 and 840

amino acids respectively. The human liver and pancreatic forms of GPI-PLD share 94.6% sequence identity. The structure of GPI-PLDs is further discussed in Scallon et al, 1991.

However, despite cloning three forms of GPI-PLD, there is no suggestion in these references as to the *in vivo* role of the enzymes. Further, the only application of the enzymes suggested is in an expression system in which a heterologous protein is expressed in a host cell as a fusion with a GPI-signal peptide, leading to the heterologous protein becoming anchored to the cell membrane by a GPI anchor, where it can be cleaved off by coexpressed or added GPI-PLD.

GPI-PLD has also been isolated from human serum by Hoener et al (1992) and this form of the enzyme was found to be identical to the human pancreatic GPI-PLD apart from changes at 531 to 534 where VIGS is replaced by MLGT. This paper also showed that treatment of serum GPI-PLD with N-glycosidase F reduced the apparent molecular weight from 123 kD to 87 kD. Similarly, by Li et al (1994) showed GPI-PLD was cleaved by trypsin into 3 fragments (2 x 40 kD and 30 kD), and by Heller et al (1994) which showed that 33, 39 and 47kD species were produced, with only the N-terminal 39 kD fragment moiety showing enzyme activity after renaturation.

It has been proposed that one function of GPI-PLD enzyme is to produce inositolphosphoglycans (IPGs) by the cleavage of "free" GPIs in the plasma membrane in response to binding of a growth factor to its receptor (Rademacher et al, 1994). This role for GPI-PLD has been demonstrated in mast cells where IgE-dependent activation of these cells results in release of their granule contents, which include substances such as histamine, a mediator of the inflammatory response. In the presence

of antigen, histamine is released; this release can be mimicked by addition of IPGs and is blocked by addition of anti-GPI-PLD antibodies (Lin et al, 1991).

The role of GPI-PLD in cleaving GPI-anchored proteins, and especially inositolphosphoglycans (IPGs), is examined in Jones et al (1997). However, the authors reflect the uncertainty in the art regarding the mechanism of IPG generation, noting that "The definitive activated enzyme, being a GPI-PLC or a GPI-PLD, has yet to be unequivocally identified" and that "little attention has been payed to the role of GPI-PLD as the hydrolysing enzyme".

In summary, despite the cloning of GPI-PLD enzymes and investigation as to their biochemical properties, the role of the enzyme *in vivo* or any possible medical use remains unknown.

Summary of the Invention

Broadly, the present invention relates to GPI-PLD for medical use, and in particular to the use of GPI-PLD in the treatment of conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD. The GPI-PLD can be the forms of the enzyme disclosed in the prior art, or the GPI-PLDs disclosed for the first time here. An example of such a condition includes septic shock which commonly occurs following abdominal surgery, severe burns, trauma or cardiac failure. Septic shock is generally preceded by a reduction in splanchnic blood flow, resulting in ischaemia and epithelial damage on reperfusion, allowing ingress of microorganisms and subsequent sepsis.

The present invetion is based on the observation that in conditions such as septic shock, endotoxin is released from the microorganisms causing sepsis, leading to the clinical symptoms of septic shock such as total organ

failure and fatal shock. The endotoxins can be glycolipids released from gram negative bacteria or glycolipids such as LAM released from mycobacteria such as Tuberculosis. Without wishing to be bound by any particular theory, these endotoxins are believed to act by inhibiting GPI-PLD.

At present, despite many attempts in the art to develop a treatment for septic shock and other related conditions, there are no approved treatments available. In particular, a reliable diagnostic test for determining whether a patient has or is at risk of developing conditions such as septic shock would be useful as an early warning of the condition and to allow timely treatment to be given.

Accordingly, in a first aspect, the present invention provides the use of GPI-PLD for the preparation of a medicament for the treatment of a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD.

In a further aspect, the present invention provides a method of treating a patient having a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising administering to the patient a therapeutically effective amount of GPI-PLD.

In the above aspects, the product of the infectious organism is typically an endotoxin, such as the glycolipids produced by gram negative or mycobacteria mentioned above.

In a further aspect, the present invention provides a pharmaceutical composition comprising a GPI-PLD protein.

In a further aspect, the present invention provides the use of GPI-PLD or IPG levels in the diagnosis of conditions mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, and especially to the diagnosis of septic shock and/or distinguishing between different forms of septic shock. By way of example, the GPI-PLD or IPG levels can be determined by measuring the amount of the material and/or a characteristic activity of the material in the biological sample.

Thus, the present invention provides a method of diagnosing a condition mediated by a product of an infectious organism, the method comprising determining the level or activity of GPI-PLD or IPGs in a biological sample from a patient. This determination can help in the diagnosis or prognosis of the patient, allowing the treatment of the patient to be tailored accordingly to the patient's individual needs. IPGs can be used in this diagnosis as the inhibition of GPI-PLD by endotoxins is likely to cause the level of IPGs (e.g. in urine, blood etc) to drop since the GPI-PLD causes the release of IPG precursors. Thus, monitoring either or both of the level of GPI-PLD or the IPGs provides a way of assessing the likelihood of developing conditions such as septic shock or their prognosis. A determination of the amount of GPI-PLD can be carried out using immobilised binding agents or by determining one or more of the activities associated with GPI-PLD and/or IPGs (see further below).

In one embodiment, the method of diagnosing a condition mediated by a product of an infectious organism which is capable of inhibiting GPI-PLD, the method comprising the steps of:

(a) contacting a biological sample obtained from the patient with a solid support having immobilised thereon a binding agent having binding sites specific for

GPI-PLD or IPGs;

(b) contacting the solid support with one or more labelled developing agents capable of binding to unoccupied binding sites, bound GPI-PLD or IPGs or occupied binding sites; and,

(c) detecting the label of the developing agents specifically binding in step (b) to obtain a value representative of the amount of GPI-PLD or IPGs in the sample.

These and other aspects of the present invention are described in more detail below.

By way of example, embodiments of the present invention will now be described in more detail with reference to the accompanying figures.

<u>Brief Description of the Figures</u>

Figure 1 shows an alignment of the deduced amino acid sequences of GPI-PLD encoded by cDNA clone A1 and the bovine and human liver GPI-PLD sequences disclosed in US Patent No: 5,418,147 (Huang et al).

Figure 2 shows the nucleic acid sequence from cDNA clone A1 aligned with the pancreatic forms of GPI-PLD disclosed in US Patent No: 5,418,147 (Huang et al) (partial sequence) and the corresponding full length nucleic acid sequence deposited at GenBank.

Figure 3 shows the amino acid sequences of the GPI-PLDs in clones a1, b2 and d3, and consist of 840, 795 and 510 amino acids respectively.

Figure 4 shows the nucleic acid sequence of cDNA clone a1 encoding GPI-PLD, consisting of 2832 bp.

Figure 5 shows the nucleic acid sequence of cDNA clone b2

encoding GPI-PLD, consisting of 2472 bp.

Figure 6 shows the nucleic acid sequence of cDNA clone d3 encoding GPI-PLD, consisting of 1942 bp.

Figure 7 shows an alignment of the deduced amino acid sequences of GPI-PLDs encoded by cDNA clones a1, b2 and d3 with the pancreatic form of the enzyme deposited at GenBank under accession number 11702.

Figure 8 shows an alignment of the nucleic acid sequences from cDNA clones a1, b2 and d3 with the cDNA sequence encoding the human pancreatic form of GPI-PLD deposited at GenBank under accession number 11702.

Detailed Description

GPI-PLD Proteins

The term "GPI-PLD biological activity" is herein defined as the enzymatic activity of GPI-PLD in cleaving the photodiester bond linking glycosylphosphatidylinositol to phosphatidic acid, e.g. releasing a GPI-anchored protein. As noted in Heller et al (1994), this activity has been localised to the N-terminal 39 kD portion of full length GPI-PLD.

The medical uses of GPI-PLD described herein can use the novel GPI-PLD variants or the forms of the enzyme disclosed in the prior art. In either event, the skilled person can use the techniques described herein and others well known in the art to produce large amounts of these proteins, or fragments or active portions thereof, for use as pharmaceuticals, in the developments of drugs and for further study into its properties and role *in vivo*.

In a further aspect of the present invention provides a polypeptide having the amino acid sequence shown in figure 3, which may be in isolated and/or purified form,

free or substantially free of material with which it is naturally associated. In one embodiment, the clone a1 has an amino acid sequence consisting of 840 amino acids, a 23 amino acid signal peptide and a 817 amino acid mature protein.

GPI-PLD proteins which are an amino acid sequence variants, alleles or derivatives can also be used in the present invention. A polypeptide which is a variant, allele or derivative may have an amino acid sequence which differs from that given in figures 1 or 3 by one or more of addition, substitution, deletion and insertion of one or more amino acids. Preferred polypeptides have GPI-PLD enzymatic function as defined above.

A GPI-PLD protein which is an amino acid sequence variant, allele or derivative of an amino acid sequence shown in figures 1 or 3 may comprise an amino acid sequence which shares greater than about 70%, greater than about 80%, greater than about 90%, greater than about 95%, greater than about 97%, greater than about 98% or greater than about 99% sequence identity with an amino acid sequence shown in figures 1 or 3. Sequence comparison and identity calculations were carried out using the Cluster program (Thompson et al, 1994), using the following parameters (Pairwise Alignment Parameters: Weight Matrix: pam series; Gap Open Penalty: 10.00; Gap Extension Penalty: 0.10). Alternatively, the GCG program could be used which is available from Genetics Computer Group, Oxford Molecular Group, Madison, Wisconsin, USA, Version 9.1. Particular amino acid sequence variants may differ from those shown in figures 1 and 3 by insertion, addition, substitution or deletion of 1 amino acid, 2, 3, 4, 5-10, 10-20 20-30, 30-50, 50-100, 100-150, or more than 150 amino acids.

The present invention also includes the use of active

portions, fragments and derivatives of the GPI-PLD proteins.

An "active portion" of GPI-PLD protein is a polypeptide which is less than said full length GPI-PLD protein, but which retains at least one its essential biological activity, e.g. the enzyme activity mentioned above. For instance, portions of GPI-PLD protein can act as sequestrators or competitive antagonists by interacting with other proteins.

A "fragment" of the GPI-PLD protein means a stretch of amino acid residues of at least about 5 to 7 contiguous amino acids, often at least about 7 to 9 contiguous amino acids, typically at least about 9 to 13 contiguous amino acids and, most preferably, at least about 20 to 30 or more contiguous amino acids.

A "derivative" of the GPI-PLD protein, or a fragment thereof, means a polypeptide modified by varying the amino acid sequence of the GPI-PLD protein, e.g. by manipulation of the nucleic acid encoding the protein or by altering the protein itself. Such derivatives of the natural amino acid sequence may involve insertion, addition, deletion or substitution of one, two, three, five or more amino acids, without fundamentally altering a biological activity of the wild type GPI-PLD protein.

A polypeptide according to the present invention may be isolated and/or purified (e.g. using an antibody) for instance after production by expression from encoding nucleic acid (for which see below). Polypeptides according to the present invention may also be generated wholly or partly by chemical synthesis. The isolated and/or purified polypeptide may be used in formulation of a composition, which may include at least one additional component, for example a pharmaceutical composition

including a pharmaceutically acceptable excipient, vehicle or carrier. A composition including a polypeptide according to the invention may be used in prophylactic and/or therapeutic treatment as discussed below.

The GPI-PLD polypeptides can also be linked to a coupling partner, e.g. an effector molecule, a label, a drug, a toxin and/or a carrier or transport molecule. Techniques for coupling the peptides of the invention to both peptidyl and non-peptidyl coupling partners are well known in the art. In one embodiment, the carrier molecule is a 16 aa peptide sequence derived from the homeodomain of *Antennapedia* (e.g. as sold under the name "Penetratin"), which can be coupled to a peptide via a terminal Cys residue. The "Penetratin" molecule and its properties are described in WO91/18981.

<u>A and P-type IPGs</u>

As mentioned above, the level of inositolphosphoglycans (IPGs) can be used in the diagnosis of conditions caused by a product of an infectious organism which is capable of inhibiting GPI-PLD, such as septic shock. Studies have shown that A-type mediators modulate the activity of a number of insulin-dependent enzymes such as cAMP dependent protein kinase (inhibits), adenylate cyclase (inhibits) and cAMP phospho-diesterases (stimulates). In contrast, P-type mediators modulate the activity of insulin-dependent enzymes such as pyruvate dehydrogenase phosphatase (stimulates), glycogen synthase phosphatase (stimulates), and cAMP dependent protein kinase (inhibits). The A-type mediators mimic the lipogenic activity of insulin on adipocytes, whereas the P-type mediators mimic the glycogenic activity of insulin on muscle. Both A-and P-type mediators inhibit cAMP dependent protein kinase and are mitogenic when added to fibroblasts in serum free media. The ability of the

mediators to stimulate fibroblast proliferation is enhanced if the cells are transfected with the EGF-receptor. A-type mediators can stimulate cell proliferation in the chick cochleovestibular ganglia.

Soluble IPG fractions having A-type and P-type activity have been obtained from a variety of animal tissues including rat tissues (liver, kidney, muscle brain, adipose, heart) and bovine liver. A-type and P-type IPG biological activity has also been detected in human liver and placenta, malaria parasitized RBC and mycobacteria. The ability of an anti-inositolglycan antibody to inhibit insulin action on human placental cytotrophoblasts and BC3H1 myocytes or bovine-derived IPG action on rat diaphragm and chick cochleovestibular ganglia suggests cross-species conservation of many structural features.

A-type substances are cyclitol-containing carbohydrates, also containing Zn^{2+} ions and optionally phosphate and having the properties of regulating lipogenic activity and inhibiting cAMP dependent protein kinase. They may also inhibit adenylate cyclase, be mitogenic when added to EGF-transfected fibroblasts in serum free medium. A-type IPGs isolated from sources such as human or bovine liver have the property of stimulating lipogenesis in adipocytes. In contrast, the A-type substances from porcine tissue disclosed herein have the properties of inhibiting lipogenesis and lowering blood glucose levels when administered to diabetics, i.e. patients or a suitable animal model.

P-type substances are cyclitol-containing carbohydrates, also containing Mn^{2+} and/or Zn^{2+} ions and optionally phosphate and having the properties of regulating glycogen metabolism and activating pyruvate dehydrogenase phosphatase. They may also stimulate the activity of glycogen synthase phosphatase, be mitogenic when added to

fibroblasts in serum free medium, and inhibit cAMP dependent protein kinase.

Methods for obtaining A-type and P-type IPGs are set out in Caro et al, 1997, and in WO98/11116 and WO98/11117.

Pharmaceutical Compositions

As mentioned above, GPI-PLD proteins and IPGs can used for treating treatment of conditions caused by a product of an infectious organism which is capable of inhibiting GPI-PLD. Thus, these materials can be formulated in pharmaceutical compositions, which may comprise, in addition to one of the above substances, a pharmaceutically acceptable excipient, carrier, buffer, stabiliser or other materials well known to those skilled in the art. Such materials should be non-toxic and should not interfere with the efficacy of the active ingredient. The precise nature of the carrier or other material may depend on the route of administration, e.g. oral, intravenous, cutaneous or subcutaneous, nasal, intramuscular, intraperitoneal routes.

Pharmaceutical compositions for oral administration may be in tablet, capsule, powder or liquid form. A tablet may include a solid carrier such as gelatin or an adjuvant. Liquid pharmaceutical compositions generally include a liquid carrier such as water, petroleum, animal or vegetable oils, mineral oil or synthetic oil. Physiological saline solution, dextrose or other saccharide solution or glycols such as ethylene glycol, propylene glycol or polyethylene glycol may be included.

For intravenous, cutaneous or subcutaneous injection, or injection at the site of affliction, the active ingredient will be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of

relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles such as sodium chloride injection, Ringer's injection, lactated Ringer's injection. Preservatives, stabilisers, buffers, antioxidants and/or other additives may be included as required.

Whether it is a polypeptide, peptide, nucleic acid molecule, small molecule or other pharmaceutically useful compound of the invention that is to be given to an individual, administration is preferably in a "prophylactically effective amount" or a "therapeutically effective amount" (as the case may be, although prophylaxis may be considered therapy), this being sufficient to show benefit to the individual. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated. Prescription of treatment, e.g. decisions on dosage etc, is within the responsibility of general practitioners and other medical doctors, and typically takes account of the disorder to be treated, the condition of the individual patient, the site of delivery, the method of administration and other factors known to practitioners. Examples of the techniques and protocols mentioned above can be found in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A. (ed), 1980.

As mentioned above, in further embodiments, the GPI-PLD can be administered alone or in combination with P and/or A-type IPGs.

GPI-PLD nucleic acid

"GPI-PLD nucleic acid" includes a nucleic acid molecule which has a nucleotide sequence encoding a polypeptide which includes the amino acid sequence shown in figures 4 to 6, and in some embodiments of the invention extends to

the known human liver and pancreatic forms of GPI-PLD (L11701 and L11702). These forms of GPI-PLD have been mapped to human chromosome 6 and are contained in the 4 centimorgan region of D6S1660-D6S1558 at positions 95.95 and 99.71 (NCBI GeneMap'98). This corresponds to the cytogenetic region of 6p21.3. This region also contains the IDDM1 and HLA loci (although the HLA genes map to the adjacent D6S1558-D6S1616 interval). The mouse GPI-PLD gene has also been mapped to chromosome 13, near the *fim 1* locus, which is found in humans on chromosome 6.

The GPI-PLD coding sequence may be that shown in figures 2, 4 to 6 or 8, a complementary nucleic acid sequence, or it may be a mutant, variant, derivative or allele of these sequences. The sequence may differ from that shown by a change which is one or more of addition, insertion, deletion and substitution of one or more nucleotides of the sequence shown. Changes to a nucleotide sequence may result in an amino acid change at the protein level, or not, as determined by the genetic code.

The encoded polypeptide may comprise an amino acid sequence which differs by one or more amino acid residues from the amino acid sequence shown in the figures. Nucleic acid encoding a polypeptide which is an amino acid sequence mutant, variant, derivative or allele of the sequence shown in figures 1, 3 or 7 is further provided by the present invention. Such polypeptides are discussed below. Nucleic acid encoding such a polypeptide may show greater than about 70% identity, greater than about 80% identity, greater than about 90% identity, greater than about 95% identity, greater than about 98% identity, or greater than about 99% identity with a sequence shown in the figures.

The present invention also includes fragments of the GPI-PLD nucleic acid sequences described herein, the

fragments preferably being at least 12, 15, 30, 45, 60, or 120 nucleotides in length.

Generally, nucleic acid according to the present invention is provided as an isolate, in isolated and/or purified form, or free or substantially free of material with which it is naturally associated, such as free or substantially free of nucleic acid flanking the gene in the human genome, except possibly one or more regulatory sequence(s) for expression. Nucleic acid may be wholly or partially synthetic and may include genomic DNA, cDNA or RNA. Where nucleic acid according to the invention includes RNA, reference to the sequence shown should be construed as reference to the RNA equivalent, with U substituted for T.

Nucleic acid sequences encoding all or part of the GPI-PLD gene and/or its regulatory elements can be readily prepared by the skilled person using the information and references contained herein and techniques known in the art (for example, see Sambrook, Fritsch and Maniatis, "Molecular Cloning, A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1989, and Ausubel et al, Short Protocols in Molecular Biology, John Wiley and Sons, 1992). These techniques include (i) the use of the polymerase chain reaction (PCR) to amplify samples of such nucleic acid, e.g. from genomic sources, (ii) chemical synthesis, or (iii) amplification in *E. coli*. Modifications to the GPI-PLD sequences can be made, e.g. using site directed mutagenesis, to provide expression of modified GPI-PLD protein or to take account of codon preference in the host cells used to express the nucleic acid.

In order to obtain expression of the GPI-PLD nucleic acid sequences, the sequences can be incorporated in a vector having control sequences operably linked to the GPI-PLD

nucleic acid to control its expression. The use of expression systems has reached an advanced degree of sophistication. The vectors may include other sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that the GPI-PLD protein is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide produced in the host cell is secreted from the cell. GPI-PLD protein can then be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the GPI-PLD protein is produced and recovering the GPI-PLD protein from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of *E. coli*, yeast, and eukaryotic cells such as COS or CHO cells. The choice of host cell can be used to control the properties of the GPI-PLD protein expressed in those cells, e.g. controlling where the polypeptide is deposited in the host cells or affecting properties such as its glycosylation and phosphorylation.

PCR techniques for the amplification of nucleic acid are described in US Patent No: 4,683,195. In general, such techniques require that sequence information from the ends of the target sequence is known to allow suitable forward and reverse oligonucleotide primers to be designed to be identical or similar to the polynucleotide sequence that is the target for the amplification. PCR comprises steps of denaturation of template nucleic acid (if double-stranded), annealing of primer to target, and polymerisation. The nucleic acid probed or used as template in the amplification reaction may be genomic DNA, cDNA or RNA. PCR can be used to amplify specific sequences from genomic DNA, specific RNA sequences and cDNA transcribed from mRNA, bacteriophage or plasmid sequences. The GPI-PLD protein nucleic acid sequences

provided herein readily allow the skilled person to design PCR primers. References for the general use of PCR techniques include Mullis et al, Cold Spring Harbor Symp. Quant. Biol., 51:263, 1987; Ehrlich (ed), PCR Technology, Stockton Press, NY, 1989; Ehrlich et al, Science, 252:1643-1650, 1991; "PCR protocols; A Guide to Methods and Applications", Eds. Innis et al, Academic Press, New York, 1990.

Nucleic acid according to the present invention is obtainable using one or more oligonucleotide probes or primers designed to hybridize with one or more fragments of the nucleic acid sequence shown in the figures, particularly fragments of relatively rare sequence, based on codon usage or statistical analysis. A primer designed to hybridize with a fragment of the nucleic acid sequence shown in the above figures may be used in conjunction with one or more oligonucleotides designed to hybridize to a sequence in a cloning vector within which target nucleic acid has been cloned, or in so-called "RACE" (rapid amplification of cDNA ends) in which cDNA's in a library are ligated to an oligonucleotide linker and PCR is performed using a primer which hybridizes with a GPI-PLD nucleic acid sequence shown in figures and a primer which hybridizes to the oligonucleotide linker.

Such oligonucleotide probes or primers, as well as the full-length sequence (and mutants, alleles, variants and derivatives) are also useful in screening a test sample containing nucleic acid for the presence of alleles, mutants and variants, especially those that lead to the production of inactive forms of GPI-PLD protein protein, the probes hybridizing with a target sequence from a sample obtained from the individual being tested. The conditions of the hybridization can be controlled to minimise non-specific binding, and preferably stringent to moderately stringent hybridization conditions are

preferred. The skilled person is readily able to design such probes, label them and devise suitable conditions for the hybridization reactions, assisted by textbooks such as Sambrook et al (1989) and Ausubel et al (1992).

Examples of "stringent conditions" are those which: (1) employ low ionic strength and high temperature for washing, for example 0.015 M sodium chloride/0.0015 M sodium citrate/0.1% sodium dodecyl sulphate at 50°C; (2) employ during hybridisation a denaturing agent, such as formamide, for example, 50% (v/v) formamide with 0.1% BSA/0.1% Ficoll/0.1% polyvinylpyrrolidone/50mM sodium phosphate buffer at pH 6.5 with 750mM sodium chloride, 75mM sodium citrate at 42°C; or (3) employ 50% formamide, 5 x SSC (0.75 M NaCl, 0.075 M sodium citrate), 50mM sodium phosphate (pH 6.8), 0.1% sodium pyrophosphate, 5 x Denhardt's solution, sonicated salmon sperm DNA (50μg/ml), 0.1% SDS, and 10% dextran sulphate at 42°C, with washes at 42°C in 0.2 x SSC and 50% formamide at 55°C, followed by high stringency wash consisting of 0.1 x SSC containing EDTA at 55°C. These hybridisation conditions may be used in the context of defining nucleic acid sequences which hybridize with GPI-PLD nucleic acid sequences.

<u>Uses of GPI-PLD Nucleic Acid</u>

The GPI-PLD nucleic acid sequences can be used in the preparation of cell lines capable of expressing GPI-PLD and in gene therapy techniques.

Thus, the present invention provides a cell line for transplantation into a patient, the cell line being transformed with nucleic acid encoding GPI-PLD, and being capable of expressing and secreting GPI-PLD. In one embodiment, the cell lines are encapsulated, e.g. in a biocompatible polymer, so that the GPI-PLD produced by the cell line can be secreted into the patient, while

preventing rejection by the immune system of the host. Methods for encapsulating cells in biocompatible polymers are described in WO93/16687 and WO96/31199.

As a further alternative, the nucleic acid encoded the GPI-PLD protein could be used in a method of gene therapy, to treat a patient who is unable to synthesize the active polypeptide or unable to synthesize it at the normal level, thereby providing the effect provided by wild-type GPI-PLD protein and suppressing the occurrence of diabetes in the target cells.

Vectors such as viral vectors have been used in the prior art to introduce genes into a wide variety of different target cells. Typically, the vectors are exposed to the target cells so that transfection can take place in a sufficient proportion of the cells to provide a useful therapeutic or prophylactic effect from the expression of the desired polypeptide. The transfected nucleic acid may be permanently incorporated into the genome of each of the targeted tumour cells, providing long lasting effect, or alternatively the treatment may have to be repeated periodically.

A variety of vectors, both viral vectors and plasmid vectors, are known in the art, see US Patent No: 5,252,479 and WO93/07282. In particular, a number of viruses have been used as gene transfer vectors, including papovaviruses, such as SV40, vaccinia virus, herpesviruses, including HSV and EBV, and retroviruses. Many gene therapy protocols in the prior art have used disabled murine retroviruses.

As an alternative to the use of viral vectors other known methods of introducing nucleic acid into cells includes electroporation, calcium phosphate co-precipitation, mechanical techniques such as microinjection, transfer

mediated by liposomes and direct DNA uptake and receptor-mediated DNA transfer.

As mentioned above, the aim of gene therapy using nucleic acid encoding the GPI-PLD protein, or an active portion thereof, is to increase the amount of the expression product of the nucleic acid in cells in which the level of the wild-type GPI-PLD protein is absent or present only at reduced levels. Target cells for gene therapy include insulin secreting β-cells or any neuron derived cells. Cell engineering can be used to provide the overexpression or repression of GPI-PLD protein in transfected cell lines which can then be subsequently transplanted to humans. Gene therapy can be employed using a promoter to drive GPI-PLD protein expression in a tissue specific manner (i.e. an insulin promoter linked to GPI-PLD cDNA will overexpress GPI-PLD protein in β-cells and transiently in the brain). If defective function of GPI-PLD protein is involved in neurological disease, GPI-PLD protein can be overexpressed in transformed cell lines for transplantation.

Gene transfer techniques which selectively target the GPI-PLD nucleic acid to target tissues are preferred. Examples of this included receptor-mediated gene transfer, in which the nucleic acid is linked to a protein ligand via polylysine, with the ligand being specific for a receptor present on the surface of the target cells.

<u>Diagnostic Methods</u>

Methods for determining the concentration of analytes in biological samples from individuals are well known in the art and can be employed in the context of the present invention to determine the presence or amount of GPI-PLD or IPGs in a biological sample from a patient. This in turn can allow a physician to determine whether a patient

suffers from or is at risk of a condition caused by a product of an infectious organism which is capable of inhibiting GPI-PLD, and so optimise the treatment of it. It mau also be possible to use this determination to distinguish between different conditions caused by products of infectious organisms.

Broadly, the methods divide into those which determine the presence or amount of GPI-PLD or IPGs in a binding assay and those which measure a characteristic activity of the GPI-PLD or IPGs.

These diagnostic methods can employ biological samples such as blood, serum, tissue samples or urine. In view of the fact that the activity of GPI-PLD is thought to be due to the level of the enzyme circulating in serum, the use of serum or blood samples is preferred.

The assay methods for determining the amount or concentration of GPI-PLD protein typically employ binding agents having binding sites capable of specifically binding to GPI-PLD in preference to other molecules. Examples of binding agents include antibodies, receptors and other molecules capable of specifically binding the enzyme. Conveniently, the binding agent(s) are immobilised on solid support, e.g. at defined locations, to make them easy to manipulate during the assay.

The sample is generally contacted with the binding agent(s) under appropriate conditions so that GPI-PLD present in the sample can bind to the binding agent(s). The fractional occupancy of the binding sites of the binding agent(s) can then be determined using a developing agent or agents. Typically, the developing agents are labelled (e.g. with radioactive, fluorescent or enzyme labels) so that they can be detected using techniques well known in the art. Thus, radioactive

labels can be detected using a scintillation counter or other radiation counting device, fluorescent labels using a laser and confocal microscope, and enzyme labels by the action of an enzyme label on a substrate, typically to produce a colour change. The developing agent(s) can be used in a competitive method in which the developing agent competes with the analyte for occupied binding sites of the binding agent, or non-competitive method, in which the labelled developing agent binds analyte bound by the binding agent or to occupied binding sites. Both methods provide an indication of the number of the binding sites occupied by the analyte, and hence the concentration of the analyte in the sample, e.g. by comparison with standards obtained using samples containing known concentrations of the analyte.

<u>Experimental</u>

The present invention is based on the realisation that GPI-PLD is responsible for the production of IPG second messengers following binding of insulin to its receptor. The IPGs then interact with other cellular enzymes instigating some of the metabolic effects of the hormone. In view of this, insulin resistance may be caused by deficiencies in GPI-PLD; it has shown that pancreatic islet cells produce and secrete GPI-PLD, which is transported in blood complexed with apolipoprotein A1, and may therefore represent the major source of circulating enzyme. If this is indeed the case then the insulin resistance seen in early type I diabetes mellitus (IDDM) may result from decreased circulating GPI-PLD levels. This may have direct therapeutic relevance in that co-infusion of insulin with GPI-PLD may in fact be a far more effective therapy for diabetic patients than insulin.

Screening of human liver cDNA library

A human liver cDNA library (Gibco BRL, cat # 10422-012,

lot # HF4703) was screened for GPI-PLD, resulting in the isolation of 3 cDNA clones. The nucleic acid sequences of the clones are shown in figures 4 to 6, with the deduced amino acid sequences shown in figure 3.

Clone a1 represents the full length cDNA. There are only two differences within the coding region of this sequence when compared to that of the human GPI-PLD pancreatic form described in the GenBank database (accession number L11702). These are a *g* to *a* conversion at positions 88 (L11702), 199 (a1) and a *t* to *g* conversion at positions 797 (L11702), 908(a1). Interestingly this latter this latter conversion creates a unique *HindIII* restriction site in the a1 clone. Both conversions result in amino acid differences, the first changes amino acid 30 from a valine in L11702 to an isoleucine in a1, and the second changes amino acid 266 from an isoleucine in L11702 to a serine in a1. Clone a1 also differs from L11702 in that it contains 5′ untranslated region (UTR) and only shares the first 168 bases of the 3′ UTR before terminating in a poly-A tail.

Clone b2 lacks the exon of GPI-PLD, which begins at position 2469 in the a1 nucleotide sequence. However, the sequence from here to the end of b2 (2444-2473) does not contain a stop codon. It is therefore not clear whether b2 represents a cDNA with a different final exon or is the produce of aberrant processing.

Clone d3 shared the coding 3′ UTR sequence of the a1 clone from a1 position 1119 onwards, however the initial 1008 base pairs of coding sequence are absent from this clone. Clone d3 contains a methionine initiation codon in frame to the coding sequence at position 202 and a unique 5′ UTR. Translation of d3 from this codon would result in a unique sequence of 6 amino acids (1-6). Clone d3 therefore appears to represent a true

transcript, in that it contains initiation and stop codons and both 5′ and 3′ UTRs. The predicted protein product of this transcript would apparently lack the catalytic domain, which has been localised to the N-terminus of the GPI-PLD enzyme (amino acids 1-375), however the 3 EF hand-like domains would still be present.

Huang et al and Tsang et al (1992) reported that two variants or isoenzymes of GPI-PLD exist, the so-called liver and pancreatic forms (accession numbers L11701 and 11702). Other workers have detected L11702 cDNAs in human breast, eye, spleen, tonsil, and pancreas, as well as in liver. However, we failed to detect the liver form of GPI-PLD in the liver or in any other tissues.

Gene mapping and localisation

The chromosomal gene isolated in the experiments above is about 20-30 kb in length. The gene was also isolated on a PAC and mapped by fluorescence-in situ hybridisation (FISH) to 6p21.3, agreeing with recent radiation hybrid maps as seen on GeneMap′98; NCBI). The IDDM1 susceptibility gene also maps to 6p21.3, although recent evidence suggests that at least two closely-linked loci for IDDM1 are in the MHC region. The MHC locus itself seems to map to a region adjoining the GPI-PLD locus rather than within the same microsatellite band, so the significance of the proximity of the GPI-PLD and IDDM1 loci is unclear.

Northern blots of the mRNA species found in liver have shown two presumed splice variants as well as the full-length transcript. One has a deletion of about 160 amino acids from the mature 817 amino acid protein. The other seems to be a C-terminal deletion, which may therefore be non-functional if other authors are correct in finding that the C-terminus is necessary for enzyme activity.

The predominant GPI-PLD species detected after tissue extraction by antibodies (Western blots) has apparent molecular weight of about 47 kD, which agrees with other authors that full-length GPI-PLD is taken up from the plasma and processed to smaller active species.

GPI-PLD obtained from serum by cells is required for second messenger signalling

The principle goal of these experiments was to determine the role of glycosylphosphatidylinositol phospholipase D (GPI-PLD) in a type one hypersensitivity reaction. This reaction involved the cross-linking of IgE receptors on the mast cell surface, leading to the release of allergic mediators.

Such an allergic reaction has been experimentally reproduced in our laboratory, using a rat basophilic leukaemia cell line, RBL-2H3. These cells naturally have unoccupied IgE receptors (FcεR1, or high-affinity receptors), allowing them to be passively sensitised with an IgE isotype of choice.

RBL-2H3 cell culture was maintained in Eagles minimum essential medium, containing 10% Foetal Bovine Serum (FBS) (heat activated), 100 U/ml Penicillin, 100 µg/ml Streptomycin and 2 mM L-glutamine.

Previous research indicates that RBL-2H3 cells derive their GPI-PLD from the culture serum (data not shown). Therefore, it follows that inactivation of this external source of GPI-PLD would deprive the cells of any further enzyme.

Inactivation of GPI-PLD activity in foetal bovine serum was achieved according to the method of Kung et al (Biochimica et Biophysica Acta, 1357:329-338, 1997). Briefly, FCS was adjusted to pH 11 using concentrated

hydrochloric acid, and incubated for 1 hour at 37°C using. After this time, the pH was adjusted to 7.4, and GPI-PLD activity was determined using an enzymatic assay (Davitz et al, J. Biol. Chem., 264:13760-13764, 1989). Results indicated that this alkaline incubation severely depleted GPI-PLD activity (data not shown).

To determine the effect of culture of RBL-2H3 cells in GPI-PLD inactive serum, the supplemented MEM was replaced with MEM in which the FBS had been inactivated. Although the cell appearance was not dramatically altered by the altered culture conditions, determination of GPI-PLD activity showed a dramatic reduction in activity.

GPI-PLD activity in cells cultured with GPI-PLD active/inactive FBS:

Active = 0.66 units GPI-PLD activity/mg of protein.

Inactive = 0.11 units GPI-PLD activity/mg of protein.

The effect of a reduced GPI-PLD activity on the cell's ability to respond to IgE cross-linking was determined as follows:

RBL-2H3 cells were grown to confluence, after which time the adherent cells were removed from the culture flask using a cell scraper. The cell density was determined, using a haemocytometer, and adjusted to 2 x 10^5 per ml. The cells were seeded at 1 ml per well in a 24 well culture plate and cultured for overnight at 37°C in a humidified 5% CO_2 incubator.

The overnight culture media was aspirated and replaced with fresh media containing Rat IgE anti-DNP 3µg/ml. After a 2 hour incubation period, the media was aspirated, and the cells were washed twice, with HEPES

Buffered Saline. Cross-linking was achieved by the addition of 200 µl of DNP-Albumin at 100 ng/ml, and incubation for 2 hours. Mediator release was determined using a colorimetric assay to detect the presence of β-hexosaminidase and compared with the total cell β-hexosaminidase content (as determined by incubation with 200 µl 5% Triton X-100 detergent). (Yasuda et al, Int. Imunol., 7:251-258, 1995). As shown in the table below, the responsiveness to cross-linking was significantly reduced in those cells that were cultured in GPI-PLD inactive media.

Percentage release in IgE linking activity assay (compared with total)

Active GPI-PLD culture = 48.79%

Inactive GPI-PLD culture = 5.07%

Phosphorylation of GPI-PLD

The phosphorylation state of the GPI-PLD enzymes can be determined using MALDI-TOF mass spectrometry as described by Yip & Hutchins (1992). Spectrums of tryptic digests of the four proteins can be compared before and after treatment with calf intestinal alkaline phosphatase. The specific kinases responsible for phosphorylation of GPI-PLD can then be determined by incubation of the GPI-PLD tryptic fragments with ATP in the presence of various kinases. Motif analysis of the amino acid sequence of human GPI-PLD using the HGMP "motif" package has revealed the presence of numerous potential phosphorylation sites for two enzymes: protein kinase C and protein kinase ck2 (formerly known as casine kinase II). These enzymes may therefore be involved in the activation of GPI-PLD. Intriguingly the activity of protein kinase ck2 has been shown to be modulated by IPGs (Alemany et al, 1990) and there is also indirect evidence suggesting that IPGs may

act through protein kinase C, thus suggesting the possibility of feedback loops regulating the production of IPGs.

GPI-PLD as a metal ion transferase

Two families of IPGs exist. IPGs of the P-type stimulate incorporation of glucose into glycogen whereas the A-type IPGs stimulate incorporation of glucose into lipid. Metal ion analysis has shown that the P-type IPGs contain manganese and the A-type zinc. It is known that the serum form of GPI-PLD contains approximately 10 atoms of zinc per molecule. Investigation can therefore show whether the different isoforms of human GPI-PLD produce IPGs with differing metal ion content.

This experiment can be performed in two ways. Firstly purified A-type and P-type IPGs can be extracted from rat liver (Caro et al, 1997) and their metal ions removed using dithiazone in chloroform. The IPGs can be incubated in the presence of radiosotopes of zinc ($^{65}Zn^{2+}$) and manganese ($^{52}Mn^{2+}$) respectively. The radiolabelled IPGs can then be added to the different isoforms of purified GPI-PLD (as determined in the above experiments) in the absence of GPI substrate thus driving the reaction from product (IPG) to substrate (GPI). It can then be determined whether or not the GPI-PLD protein have incorporated radioactive metal ions from the IPGs. The reverse situation will also be examined, whereby the metal ions of GPI-PLD isoforms are replaced by the respective radioisotopes. GPI-PLD can then be incubated with GPIs extracted from membrane preparations and the resulting IPG products analysed for incorporation of radioisotope. These experiments will thus determine whether or not GPI-PLD is responsible for the transfer of divalent cations (Mn^{2+} or Zn^{2+}) to its IPG products.

Site of action

The function of the enzyme in releasing GPI-anchored proteins, and its postulated function as the generator of IPG second messengers require the enzyme to be active at the cell surface. It is known that GPI-anchored proteins accumulate in clusters in caveolae, an uncoated pit membrane specialisation, and so this is a good potential site for GPI-PLD activity. Analysis of the primary structure of the protein predicts a secondary structural arrangement of four amphipathic helices, thus suggesting that the protein can interact with lipids in membranes. Previous experiments have demonstrated significant amounts of the enzyme in the lyososmal fraction but not in the cytosol. The location of GPI-PLD will be examined by staining tissues with anti-GPI-PLD antibodies, followed by a gold particle-labelled second antibody. Tissue can then be prepared for transmission electron microscopy and the location of the GPI-PLD protein determined. Caveolae will also be produced according to the protocol of Chang et al (1994), which involves three rounds of sucrose step gradient ultracentrifugation. Caveolae-enriched proteins will then be separated by SDS-PAGE and electrophorectically transferred to nitrocellulose membranes. We can then use the anti-GPI-PLD antibody to determine if GPI-PLD is present in these membrane specialisations.

Activation of GPI-PLD

If GPI-PLD is found to be phosphorylated by protein kinase C and/or protein kinase ck2 by MALDI-TOF spectrometry, the interaction of these proteins can be confirmed using immunoprecipitation since antibodies to GPI-PLD, protein kinase C and protein kinase ck2 have all be produced. The yeast two hybrid system can also be used to identify other proteins which interact with GPI-PLD in the cell. The yeast two hybrid systems (Chen et al, 1991) is based on the property of the yeast

transcriptional activator Ga14, which is separable into DNA binding and transcriptional activating domains. GPI-PLD cDNAs can be cloned in frame into the DNA binding domain vector. This will be co-transfected into an appropriate yeast host strain along with a library of cDNAs cloned into the activation domain vector. Interaction of a protein with GPI-PLD will therefore result in localisation of the activation and DNA binding domains, and hence transcription of the galactosidase reporter gene. Clones containing interacting proteins are then identified by the colour reaction they produce. The advantage of this system is that the gene encoding the interacting protein is immediately available for sequence analysis and thus identification. The use of this system has enabled identification of many interacting proteins and the system available in kit form from Clontech. This also provides a method of screening for sustances which are capable of activating GPI-PLD, e.g. for further development as lead compounds.

Discussion

GPI-PLD is a metalloenzyme with 5 and 10 atoms per molecule of calcium and zinc, respectively. It circulates in a complex with apolipoprotein A1. GPI-PLD is produced in the pancreas by both α and β-cells in the islets of Langerhans. It is also produced by a mouse insulinoma cell line (TC3), with GPI-PLD and insulin generally colocalised intracellularly. The enzyme was shown to be secreted in response to insulin secretagogues. Both isoenzymes of GPI-PLD also seem to be present in liver; a major part of the activity could be washed away from the tissue by extraction with detergent-free buffer (thus, likely to be the plasma enzyme). There is some suggestions that the liver, as well as the pancreas, may contribute to the serum pool of GPI-PLD as patients with liver disease have lower levels of active enzyme, which is correlated with the reduced

albumin levels.

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The references mentioned herein are all incorporated by reference in their entirety.

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Figure 1: Alignment of GPI-PLD deduced amino acid sequences

Top: protein produced from cDNA clone A1
Mid: protein produced from Roche patent bovine liver sequence
Bot: protein produced from Roche patent human liver sequence

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MSAFRLWPGLLIMLG-SLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDA
MSAFRFWSGLLMLLG-FLCPRSSPCGISTHIEIGHRALEFLHLQDGSINYKELLLRHQDA
MSAFRLWPGLLMIVMASLCHRGSSCGLSTHIEIGHRALEFLHLHNGHVNYKELLLEHQDA

YQAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFL
YQAGSVFPDSFYPSICERGQFHDVSESTHWTPFLNASVHYIRKNYPLPWDEDTEKLVAFL
YQAGTVFPDCFYPSLCKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFL

FGITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLA
FGITSHMVADVNWHSLGIENGFLRTMAAIDFHNSYPEAHPAGDFGGDVLSQFEFKFNYLS
FGITSHMVADVSWHSLGIEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLA

RRWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFL
RHWYVPAEDLLGIYRELYGRIVITKKAIVDCSYLQFLEMYAEMLAISKLYPTYSVKSPFL
RRWYVPVKDLLGIYEKLYGREVITENVIVDCSHIQFLEMYGEMLAVSKLYPSYSTKSPFL

VEQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPENPLFIACGGQQNHTQG
VEQFQEYFLGGLEDMAFWSTNIYHLTSTMLKNGTSNCNLPENP---LFITCGGQQNNTHG
VEQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCSLFENPENPLFIACGGQQNHTQG

SKMQKNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIG
SKVQKNGFHKNVTAALTKNIGKHINYTKRGVFFSVDSWTMDFLSFMYKSLERSIREMFIG
SKMQKNDFHRNLTSSLTENIDRNINYTERGVFFSVNSWTPDSMSFIYKALERNVRTMFIG

GSQLSQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRV
SSQP-LTHVSSPAASYYLSFPYTRLGWAMTSADLNQDGYGDLVVGAPGYSHPGRIHVGRV
GSQLSQKHISSPLASYFLSFPYARLGWAMTSADLNQDGYGDLVVGAPGYSRPGRIHIGRV

YLIYGNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGS
YLIYGNDLG-PRIDLDLDKEAHGILEGFQPSGRFGSAVAVLDFNVDGVPDLAVGAPSVGS
YLIYGNELGLPPVDLDLDKEAHGILEGFQPSGRFGSALAMLDFNMDGVPDLAVGAPSVGS

EQLTYKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPD-LVIGSP
EKLTYTGAVYVYFGSKQGQLSSSPNVTISCQDTYCNLGWTLLAADVDGDSEPDLFVIGSP
EQLTYKGAVYVYFGSKQGRMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPD-LVIGSP

FAPGGGKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLL
FAFGGGKQKGIVAAFYSGSSYSSREKLNVEAANWMVKGEEDFAWLGYSLHGVNVNNRTLL
FAPGGGKQKGIVAAFYSGPSLSNKEKLNVEAANWTVRGEEDFAWFGYSLHGVTVDNRTLL

LVGSPTWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGH
LAGSPTWKDTSSQGHLFRTRDEKQSPGRVYGYFPPICQSWFTISGDKAMGKLGTSLSSGH
LVGSPTWKNASRLGRLLHIRDEKKSLGRVYGYFPPNSQSWFTIVGDKAMGKLGTSLSSGH

VLMNGTLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRF
VIVNGTRTQVLLVGAPTQDVVSKS-FLTMTLHQGGSTRMYELTPDSQPSLLSTFSGNRRF
VLMNGTLTQVLLVGAPTRDDVSKMAFLTMTLHQGGATRMYALTSDLQPPLLSTFSGDRRF

SRFGGVLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKC
SRFGGVLHLSDLDNDGLDEIIVAAPLRITDATAGLMGEEDGRVYVFNGKQITVGDVTGKC
SRFGGVLHLSDLDDDGVDEIIVAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKC

KSWITPCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVY
KSWVTPCPEEKAQYVLISPEAGSRFGSSVITVRSKEKNQVIIAAGRSSLGARLSGVLHIY
KSWMTPCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVY
```

SLGSD
RLGQD
SLGSD

2.1

Figure 2: Alignment of human GPI-PLD nucleic acid sequences

Top: pancreatic-form cDNA sequence from GenBank database
mid: our sequence cloned from human liver cDNA library
bot: Roche patent pancreatic-form partial cDNA sequence

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       ------------------------------------------------------------
1      GTGACCTGCTTAGAGAGAAGCGGTGGGTCTGCACCTGGATTTTGGAGTCCCAGTGCTGCT   60
       ------------------------------------------------------------

       ---------------------------------------------------ATGTCTGCT    9
1      ------------------------------------------------------------
61     GCAGCTCTGAGCATTCCCACGTCACCAGAGAAGCCGGTGGGCAATGAGAGCATGTCTGCT  120
       ------------------------------------------------------------

10     TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG   69
121    TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG  180
       ------------------------------------------------------------

70     TGTGGCCTTTCAACACACGTAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC  129
181    TGTGGCCTTTCAACACACATAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC  240
       ------------------------------------------------------------

130    AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA  189
241    AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA  300
       ------------------------------------------------------------

190    ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG  249
301    ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG  360
       ------------------------------------------------------------

250    TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC  309
361    TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC  420
       ------------------------------------------------------------

310    TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT  369
421    TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT  480
       ------------------------------------------------------------

370    TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG  429
481    TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG  540
       ------------------------------------------------------------

430    ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT  489
541    ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT  600
       ------------------------------------------------------------

490    GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT  549
601    GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT  660
       ------------------------------------------------------------

550    GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC  609
661    GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC  720
       ------------------------------------------------------------

610    GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA  669
721    GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA  780
       ------------------------------------------------------------

670    GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC  729
781    GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC  840
       ------------------------------------------------------------
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730   CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT  789
841   CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT  900
      ------------------------------------------------------------

790   CTAACAATCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG  849
901   CTAACAAGCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG  960
      ------------------------------------------------------------

850   TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT  909
961   TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT  1020
      ------------------------------------------------------------

910   TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT  969
1021  TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT  1080
      ------------------------------------------------------------

970   GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC  1029
1081  GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC  1140
      ------------------------------------------------------------

1030  AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG  1089
1141  AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG  1200
      ------------------------------------------------------------

1090  CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG  1149
1201  CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG  1260
      ------------------------------------------------------------

1150  GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA  1209
1261  GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA  1320
      ------------------------------------------------------------

1210  GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC  1269
1321  GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC  1380
      ------------------------------------------------------------

1270  CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC  1329
1381  CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC  1440
      ------------------------------------------------------------

1330  TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC  1389
1441  TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC  1500
      ------------------------------------------------------------

1390  GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT  1449
1501  GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT  1560
      ------------------------------------------------------------

1450  GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC  1509
1561  GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC  1620
      ------------------------------------------------------------

1510  ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT  1569
1621  ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT  1680
      ------------------------------------------------------------

1570  GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG  1629
1681  GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG  1740
      ------------------------------------------------------------

1630  AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC  1689
1741  AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC  1800
1     -------------------------CTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC  35
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1690 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 1749
1801 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 1860
36   GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 95

1750 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG 1809
1861 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG 1920
96   CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGGAGCCCGACCTGGAAG 155

1810 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1869
1921 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1980
156  AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 215

1870 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 1929
1981 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 2040
216  GTGTATGGCTACTTCC-ACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 275

1930 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 1989
2041 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 2100
276  ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGTCACGTACTGATGAATGGGACTCTGAAA 335

1990 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 2049
2101 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 2160
336  CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 395

2050 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 2109
2161 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 2220
396  GTGACCCTACACCAAGGCGGAGCCACTCGCGTGTACGCACTCATATCTGACGCGCAGCCT 455

2110 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 2169
2221 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 2280
456  CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 515

2170 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 2229
2281 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 2340
516  TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 575

2230 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 2289
2341 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 2400
576  GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 635

2290 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 2349
2401 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 2460
636  AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 695

2350 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 2409
2461 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 2520
696  GAAGAAAAGGCGCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 755

2410 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 2469
2521 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 2580
756  CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 815

2470 TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT 2529
2581 TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT 2640
816  TTGGGAGCCCGACTCTCCGGGGCACTTCACGTCTATAGCCTTGGCTCAGATTGAAGATTT 875

2530 CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT 2589
2641 CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT 2700
876  CACTGCATTTCCCCACTCTGCCCACCTCTCTCATGCTGAATCACATCCATGGTGAGCATT 935

2590 TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC 2649
2701 TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC 2760
936  TTGATGGACAAAGTGGCACATCCAGTGGAGCGGTGGTAGATCCTGATAGACATGGGGCTC 995
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2650 CTGGGA------------------------------------------------------ 2655
2761 CTGGGA------------------------------------------------------ 2766
996  CTGGGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCA 1055

2656 ----------------------------GTAGAGAGACACACTAACAGCCACACCCTCTG 2687
2767 ----------------------------GTAGAGAGACACACTAACAGCCACACCCTCTG 2798
1056 GAAGGGAATTGTGGCTGCGTTTTATTGAGTAGAGAGACACACTAACAGCCACACCCTCTG 1115

2688 GAAATCTGATACAGTAAATATATGACTGCACCAGAAATATGTGAAATAGCAGACATTCTG 2747
2799 GAAATCTGATACAGTAAATATATGACTGCACCAG-------------------------- 2833
1116 GAAATCTGATACAGTAAATATATGACTACACCAGAAATATGTGAAATAGCAGACATTCTG 1175

2748 CTTACTCATGTCTCCTTCCACAGTTTACTTCCTCGCTCCCTTTGCATCTAAACCTTTCTT 2807
     ------------------------------------------------------------
1176 CTTACTCATGTCTCCTTCCACAGTTTACTTCCTCGCTCCCTTTGCATCTAAACCTTTCTT 1235

2808 CTTTCCCAACTTATTGCCTGTAGTCAGACCTGCTGTACAACCTATTTCCTCTTCCTCTTG 2867
     ------------------------------------------------------------
1236 CTTTCCCAACTTATTGCCTGTAGTC----------------------------------- 1261

2868 AATGTCTTTCCAGTGGCTGGAAAGGTCCCTCTGTGGTTATCTGTTAGAACAGTCTCTGTA 2927
     ------------------------------------------------------------
     ------------------------------------------------------------

2928 CACAATTCCTCCTAAAAACATCCTTTTTTAAAAAAAGAATTGTTCAGCCATAAAGAAAGA 2987
     ------------------------------------------------------------
     ------------------------------------------------------------

2988 ACAAGATCATGCCCTTTGCAGGGACATGGATGGAGCTGGAGGCCATTATCCTTCATAAAC 3047
     ------------------------------------------------------------
     ------------------------------------------------------------

3048 TATTGCAGGAACAGAAAACCAAACACTCCATATTCTCACTTGTAAGTGGGAGCTAAGTGA 3107
     ------------------------------------------------------------
     ------------------------------------------------------------

3108 GAACACGTGGACACATAGAGGGAAACAACACACACTGGGGCCTATGAGAGGGCGGAAGGT 3167
     ------------------------------------------------------------
     ------------------------------------------------------------

3168 GGGAGGAGGGAGAGATCAGGAAAAATAACTAATGGATACTTAGGGTGATGAAATAATCTG 3227
     ------------------------------------------------------------
     ------------------------------------------------------------

3228 TGTAACAAACCCCCATGACACACCTTTATGTATGTAACAAACCAGCACTTCCTGCGCATG 3287
     ------------------------------------------------------------
     ------------------------------------------------------------

3288 TACCCCTGAACTTAAAAGTTAAAAAAAAGTTGAACTTAAAAATAACAGATTGGCCCATGC 3347
     ------------------------------------------------------------
     ------------------------------------------------------------

3348 CAATCAAAGTATAATAGAAAGCATAGTATAC 3378
     -------------------------------
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Figure 3: Amino acid sequences of GPI-PLD a1, b2 and d3.

cDNA clone d3

MILLFQDSMSFIYKALERNIRTMFIGGSQLSQKHVSSPLASYFLSFPYARLGWAMTSADL
NQDGHGDLVVGAPGYSRPGHIHIGRVYLIYGNDLGLPPVDLDLDKEAHRILEGFQPSGRF
GSALAVLDFNVDGVPDLAVGAPSVGSEQLTKGAVYVYFGSKQGGMSSSPNITISCQDIYC
NLGWTLLAADVNGDSEPDLVIGSPFAPGGGKQKGIVAAFYSGPSLSDKEKLNVEAANWTV
RGEEDFSWFGYSLHGVTVDNRTLLLVGSPTWKNASRLGHLLHIRDEKKSLGRVYGYFPPN
GQSWFTISGDKAMGKLGTSLSSGHVLMNGTLKQVLLVGAPTYDDVSKVAFLTVTLHQGGA
TRMYALISDAQPLLLSTFSGDRRFSRFGGVLHLSDLDDDGLDEIIMAAPLRIADVTSGLI
GGEDGRVYVYNGKETTLGDMTGKCKSWITPCPEEKAQYVLISPEASSRFGSSLITVRSKA
KNQVVIAAGRSSLGARLSGALHVYSLGSD

cDNA clone b2

MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY
QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF
GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR
RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV
EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ
KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL
SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY
GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT
YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG
GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP
TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG
TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG
VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT
PCPEEKVSEKKKKKK

cDNA clone a1

MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY
QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF
GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR
RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV
EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ
KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL
SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY
GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT
YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG
GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP
TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG
TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG
VLHLSDLDDDGLDEIIMAAPLRIADVTSGLIGGEDGRVYVYNGKETTLGDMTGKCKSWIT
PCPEEKAQYVLISPEASSRFGSSLITVRSKAKNQVVIAAGRSSLGARLSGALHVYSLGSD

Figure 4: Human GPI-PLD cDNA clone a1

2832 bp: 690 a 688 c 735 g 719 t

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   1 gtgacctgct tagagagaag cggtgggtct gcacctggat tttggagtcc cagtgctgct
  61 gcagctctga gcattcccac gtcaccagag aagccggtgg gcaatgagag catgtctgct
 121 ttcaggttgt ggcctggcct gctgatcatg ttgggttctc tctgccatag aggttcaccg
 181 tgtggccttt caacacacat agaaatagga cacagagctc tggagtttct tcagcttcac
 241 aatgggcgtg ttaactacag agagctgtta ctagaacacc aggatgcgta tcaggctgga
 301 atcgtgtttc ctgattgttt ttaccctagc atctgcaaag gaggaaaatt ccatgatgtg
 361 tctgagagca ctcactggac tccgtttctt aatgcaagcg ttcattatat ccgagagaac
 421 tatcccCttc cctgggagaa ggacacagag aaactggtag ctttcttgtt tggaattact
 481 tctcacatgg cggcagatgt cagctggcat agtctgggcc ttgaacaagg attccttagg
 541 accatgggag ctattgattt tcacggctcc tattcagagg ctcattcggc tggtgatttt
 601 ggaggagatg tgttgagcca gtttgaattt aattttaatt accttgcacg acgctggtat
 661 gtgccagtca aagatctact gggaatttat gagaaactgt atggtcgaaa agtcatcacc
 721 gaaaatgtaa tcgttgattg ttcacatatc cagttcttag aaatgtatgg tgagatgcta
 781 gctgtttcca agttatatcc cacttactct acaaagtccc cgtttttggt ggaacaattc
 841 caagagtatt ttcttggagg actggatgat atggcatttt ggtccactaa tatttaccat
 901 ctaacaagct tcatgttgga gaatgggacc agtgactgca acctgcctga gaaccctctg
 961 ttcattgcat gtggcggcca gcaaaaccac acccagggct caaaaatgca gaaaaatgat
1021 tttcacagaa atttgactac atccctaact gaaagtgttg acaggaatat aaactatact
1081 gaaagaggag tgttctttag tgtaaattcc tggacccCgg attccatgtc ctttatctac
1141 aaggctttgg aaaggaacat aaggacaatg ttcataggtg gctctcagtt gtcacaaaag
1201 cacgtctcca gccccttagc atcttacttc ttgtcatttc cttatgcgag gcttggctgg
1261 gcaatgacct cagctgacct caaccaggat gggcacggtg acctcgtggt gggcgcacca
1321 ggctacagcc gccccggcca catccacatc gggcgcgtgt acctcatcta cggcaatgac
1381 ctgggcctgc cacctgttga cctggacctg gacaaggagg cccacaggat ccttgaaggc
1441 ttccagccct caggtcggtt tggctcggcc ttggctgtgt tggactttaa cgtggacggc
1501 gtgcctgacc tggccgtggg agctccctcg gtgggctccg agcagctcac ctacaaaggt
1561 gccgtgtatg tctactttgg ttccaaacaa ggaggaatgt cttcttcccc taacatcacc
1621 atttcttgcc aggacatcta ctgtaacttg ggctggactc tcttggctgc agatgtgaat
1681 ggagacagtg aacccgatct ggtcatcggc tccccttttg caccaggtgg agggaagcag
1741 aagggaattg tggctgcgtt ttattctggc cccagcctga gcgacaaaga aaaactgaac
1801 gtggaggcag ccaactggac ggtgagaggc gaggaagact tctcctggtt tggatattcc
1861 cttcacggtg tcactgtgga caacagaacc ttgctgttgg ttgggagccc gacctggaag
1921 aatgccagca ggctgggcca tttgttacac atccgagatg agaaaaagag ccttgggagg
1981 gtgtatggct acttcccacc aaacggccaa agctggttta ccatttctgg agacaaggca
2041 atggggaaac tgggtacttc cctttccagt ggccacgtac tgatgaatgg gactctgaaa
2101 caagtgctgc tggttggagc ccctacgtac gatgacgtgt ctaaggtggc attcctgacc
2161 gtgaccctac accaaggcgg agccactcgc atgtacgcac tcatatctga cgcgcagcct
2221 ctgctgctca gcaccttcag cggagaccgc cgcttctccc gatttggtgg cgttctgcac
2281 ttgagtgacc tggatgatga tggcttagat gaaatcatca tggcagcccc cctgaggata
2341 gcagatgtaa cctctggact gattgggggа gaagacggcc gagtatatgt atataatggc
2401 aaagagacca ccccttggtga catgactggc aaatgcaaat catggataac tccatgtcca
2461 gaagaaaagg cccaatatgt attgatttct cctgaagcca gctcaaggtt tgggagctcc
2521 ctcatcaccg tgaggtccaa ggcaaagaac caagtcgtca ttgctgctgg aaggagttct
2581 ttgggagccc gactctccgg ggcacttcac gtctatagcc ttggctcaga ttgaagattt
2641 cactgcattt ccccactctg cccacctctc tcatgctgaa tcacatccat ggtgagcatt
2701 ttgatggaca aagtggcaca tccagtggag cggtggtaga tcctgataga catggggctc
2761 ctgggagtag agagacacac taacagccac accctctgga aatctgatac agtaaatata
2821 tgactgcacc ag
```

Figure 5: Human GPI-PLD cDNA clone b2

2472 bp: 617 a 588 c 639 g 628 t

```
   1 gtctgcacct ggattttgga gtcccagtgc tgctgcagct ctgagcattc ccacgtcacc
  61 agagaagccg gtgggcaatg agagcatgtc tgctttcagg ttgtggcctg gcctgctgat
 121 catgttgggt tctctctgcc atagaggttc accgtgtggc ctttcaacac acatagaaat
 181 aggacacaga gctctggagt ttcttcagct tcacaatggg cgtgttaact acagagagct
 241 gttactagaa caccaggatg cgtatcaggc tggaatcgtg tttcctgatt gtttttaccc
 301 tagcatctgc aaaggaggaa aattccatga tgtgtctgag agcactcact ggactccgtt
 361 tcttaatgca agcgttcatt atatccgaga gaactatccc cttccctggg agaaggacac
 421 agagaaactg gtagctttct tgtttggaat tacttctcac atggcggcag atgtcagctg
 481 gcatagtctg ggccttgaac aaggattcct taggaccatg ggagctattg attttcacgg
 541 ctcctattca gaggctcatt cggctggtga ttttggagga gatgtgttga gccagtttga
 601 atttaatttt aattaccttg cacgacgctg gtatgtgcca gtcaaagatc tactgggaat
 661 ttatgagaaa ctgtatggtc gaaaagtcat caccgaaaat gtaatcgttg attgttcaca
 721 tatccagttc ttagaaatgt atggtgagat gctagctgtt tccaagttat atcccactta
 781 ctctacaaag tccccgtttt tggtggaaca attccaagag tattttcttg gaggactgga
 841 tgatatggca ttttggtcca ctaatattta ccatctaaca agcttcatgt tggagaatgg
 901 gaccagtgac tgcaacctgc ctgagaaccc tctgttcatt gcatgtggcg gccagcaaaa
 961 ccacacccag ggctcaaaaa tgcagaaaaa tgattttcac agaaatttga ctacatccct
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1081 ttcctggacc ccggattcca tgtcctttat ctacaaggct ttggaaagga acataaggac
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1381 cctggacaag gaggcccaca ggatccttga aggcttccag ccctcaggtc ggtttggctc
1441 ggccttggct gtgttggact ttaacgtgga cggcgtgcct gacctggccg tgggagctcc
1501 ctcggtgggc tccgagcagc tcacctacaa aggtgccgtg tatgtctact ttggttccaa
1561 acaaggagga atgtcttctt cccctaacat caccatttct tgccaggaca tctactgtaa
1621 cttgggctgg actctcttgg ctgcagatgt gaatggagac agtgaacccg atctggtcat
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1741 tggccccagc ctgagcgaca aagaaaaact gaacgtggag gcagccaact ggacggtgag
1801 aggcgaggaa gacttctcct ggtttggata ttcccttcac ggtgtcactg tggacaacag
1861 aaccttgctg ttggttggga gcccgacctg gaagaatgcc agcaggctgg gccatttgtt
1921 acacatccga gatgagaaaa agagccttgg gagggtgtat ggctacttcc caccaaacgg
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2101 gtacgatgac gtgtctaagg tggcattcct gaccgtgacc ctacaccaag gcggagccac
2161 tcgcatgtac gcactcatat ctgacgcgca gcctctgctg ctcagcacct tcagcggaga
2221 ccgccgcttc tcccgatttg gtggcgttct gcacttgagt gacctggatg atgatggctt
2281 agatgaaatc atcatggcag ccccccctgag gatagcagat gtaacctctg gactgattgg
2341 gggagaagac ggccgagtat atgtatataa tggcaaagag accacccttg gtgacatgac
2401 tggcaaatgc aaatcatgga taactccatg tccagaagaa aaggtaagtg aaaaaaaaaa
2461 aaaaaaaaaa aa
```

Figure 6: Human GPI-PLD cDNA clone d3

1942 bp: 455 a 496 c 502 g 489 t

```
   1 gggctgtaac tctgccatcc ctcagcataa tttgggggta tgatttcact atcctaattg
  61 cctgtcctaa gtgatcttac ttgctgatag gacctaatgt tttattttat tgtttagcac
 121 ttctaaaaac tcatttcctt tacacaagtc caatactttg gacaggaaac agtagctttg
 181 ttgattatgc tacgtgtctt tactgtctat aatgattctt ttatttcagg attccatgtc
 241 ctttatctac aaggctttgg aaaggaacat aaggacaatg ttcataggtg gctctcagtt
 301 gtcacaaaag cacgtctcca gcccttagc atcttacttc ttgtcatttc cttatgcgag
 361 gcttggctgg gcaatgacct cagctgacct caaccaggat gggcacggtg acctcgtggt
 421 gggcgcacca ggctacagcc gccccggcca catccacatc gggcgcgtgt acctcatcta
 481 cggcaatgac ctgggcctgc cacctgttga cctggacctg gacaaggagg cccacaggat
 541 ccttgaaggc ttccagccct caggtcggtt tggctcggcc ttggctgtgt tggactttaa
 601 cgtggacggc gtgcctgacc tggccgtggg agctccctcg gtgggctccg agcagctcac
 661 ctacaaaggt gccgtgtatg tctactttgg ttccaaacaa ggaggaatgt cttcttcccc
 721 taacatcacc atttcttgcc aggacatcta ctgtaacttg ggctggactc tcttggctgc
 781 agatgtgaat ggagacagtg aacccgatct ggtcatcggc tccccttttg caccaggtgg
 841 agggaagcag aagggaattg tggctgcgtt ttattctggc cccagcctga gcgacaaaga
 901 aaaactgaac gtggaggcag ccaactggac ggtgagaggc gaggaagact tctcctggtt
 961 tggatattcc cttcacggtg tcactgtgga caacagaacc ttgctgttgg ttgggagccc
1021 gacctggaag aatgccagca ggctgggcca tttgttacac atccgagatg agaaaaagag
1081 ccttgggagg gtgtatggct acttcccacc aaacggccaa agctggttta ccatttctgg
1141 agacaaggca atgggggaaac tgggtacttc cctttccagt ggccacgtac tgatgaatgg
1201 gactctgaaa caagtgctgc tggttggagc ccctacgtac gatgacgtgt ctaaggtggc
1261 attcctgacc gtgaccctac accaaggcgg agccactcgc atgtacgcac tcatatctga
1321 cgcgcagcct ctgctgctca gcaccttcag cggagaccgc cgcttctccc gatttggtgg
1381 cgttctgcac ttgagtgacc tggatgatga tggcttagat gaaatcatca tggcagcccc
1441 cctgaggata gcagatgtaa cctctggact gattgggga gaagacggcc gagtatatgt
1501 atataatggc aaagagacca cccttggtga catgactggc aaatgcaaat catggataac
1561 tccatgtcca gaagaaaagg cccaatatgt attgatttct cctgaagcca gctcaaggtt
1621 tgggagctcc ctcatcaccg tgaggtccaa ggcaaagaac caagtcgtca ttgctgctgg
1681 aaggagttct ttgggagccc gactctccgg ggcacttcac gtctatagcc ttggctcaga
1741 ttgaagattt cactgcattt ccccactctg cccacctctc tcatgctgaa tcacatccat
1801 ggtgagcatt ttgatggaca aagtggcaca tccagtggag cggtggtaga tcctgataga
1861 catggggctc ctgggagtag agagacacac taacagccac accctctgga aatctgatac
1921 agtaaatata tgactgcacc ag
```

7.1

Figure 7: Alignment of GPIPLD protein sequences

```
database  MSAFRLWPGLLIMLGSLCHRGSPCGLSTHVEIGHRALEFLQLHNGRVNYRELLLEHQDAY   60
d3        ------------------------------------------------------------
b2        MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY   60
a1        MSAFRLWPGLLIMLGSLCHRGSPCGLSTHIEIGHRALEFLQLHNGRVNYRELLLEHQDAY   60

database  QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF  120
d3        ------------------------------------------------------------
b2        QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF  120
a1        QAGIVFPDCFYPSICKGGKFHDVSESTHWTPFLNASVHYIRENYPLPWEKDTEKLVAFLF  120

database  GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR  180
d3        ------------------------------------------------------------
b2        GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR  180
a1        GITSHMAADVSWHSLGLEQGFLRTMGAIDFHGSYSEAHSAGDFGGDVLSQFEFNFNYLAR  180

database  RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV  240
d3        ------------------------------------------------------------
b2        RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV  240
a1        RWYVPVKDLLGIYEKLYGRKVITENVIVDCSHIQFLEMYGEMLAVSKLYPTYSTKSPFLV  240

database  EQFQEYFLGGLDDMAFWSTNIYHLTIFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ  300
d3        ------------------------------------------------------------
b2        EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ  300
a1        EQFQEYFLGGLDDMAFWSTNIYHLTSFMLENGTSDCNLPENPLFIACGGQQNHTQGSKMQ  300

database  KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL  360
d3        ------------------------------MILLFQDSMSFIYKALERNIRTMFIGGSQL   30
b2        KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL  360
a1        KNDFHRNLTTSLTESVDRNINYTERGVFFSVNSWTPDSMSFIYKALERNIRTMFIGGSQL  360

database  SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY  420
d3        SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY   90
b2        SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY  420
a1        SQKHVSSPLASYFLSFPYARLGWAMTSADLNQDGHGDLVVGAPGYSRPGHIHIGRVYLIY  420

database  GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  480
d3        GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  150
b2        GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  480
a1        GNDLGLPPVDLDLDKEAHRILEGFQPSGRFGSALAVLDFNVDGVPDLAVGAPSVGSEQLT  480

database  YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  540
d3        YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  210
b2        YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  540
a1        YKGAVYVYFGSKQGGMSSSPNITISCQDIYCNLGWTLLAADVNGDSEPDLVIGSPFAPGG  540

database  GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  600
d3        GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  270
b2        GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  600
a1        GKQKGIVAAFYSGPSLSDKEKLNVEAANWTVRGEEDFSWFGYSLHGVTVDNRTLLLVGSP  600

database  TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  660
d3        TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  330
b2        TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  660
a1        TWKNASRLGHLLHIRDEKKSLGRVYGYFPPNGQSWFTISGDKAMGKLGTSLSSGHVLMNG  660

database  TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  720
d3        TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  390
b2        TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  720
a1        TLKQVLLVGAPTYDDVSKVAFLTVTLHQGGATRMYALISDAQPLLLSTFSGDRRFSRFGG  720
```

Figure 8: Alignment of human GPI-PLD nucleic acid sequences

1: pancreatic-form: cDNA sequence from GenBank database (L11702)
2: cDNA clone A1
3: cDNA clone B2
4: cDNA clone D3

```
        ------------------------------------------------------------
1       GTGACCTGCTTAGAGAGAAGCGGTGGGTCTGCACCTGGATTTTGGAGTCCCAGTGCTGCT   60
1       --------------------------GTCTGCACCTGGATTTTGGAGTCCCAGTGCTGCT   34
        ------------------------------------------------------------

1       ---------------------------------------------------ATGTCTGCT    9
61      GCAGCTCTGAGCATTCCCACGTCACCAGAGAAGCCGGTGGGCAATGAGAGCATGTCTGCT  120
35      GCAGCTCTGAGCATTCCCACGTCACCAGAGAAGCCGGTGGGCAATGAGAGCATGTCTGCT   94
        ------------------------------------------------------------

10      TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG   69
121     TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG  180
95      TTCAGGTTGTGGCCTGGCCTGCTGATCATGTTGGGTTCTCTCTGCCATAGAGGTTCACCG  154
        ------------------------------------------------------------

70      TGTGGCCTTTCAACACACGTAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC  129
181     TGTGGCCTTTCAACACACATAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC  240
155     TGTGGCCTTTCAACACACATAGAAATAGGACACAGAGCTCTGGAGTTTCTTCAGCTTCAC  214
        ------------------------------------------------------------

130     AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA  189
241     AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA  300
215     AATGGGCGTGTTAACTACAGAGAGCTGTTACTAGAACACCAGGATGCGTATCAGGCTGGA  274
        ------------------------------------------------------------

190     ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG  249
301     ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG  360
275     ATCGTGTTTCCTGATTGTTTTTACCCTAGCATCTGCAAAGGAGGAAAATTCCATGATGTG  334
        ------------------------------------------------------------

250     TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC  309
361     TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC  420
335     TCTGAGAGCACTCACTGGACTCCGTTTCTTAATGCAAGCGTTCATTATATCCGAGAGAAC  394
        ------------------------------------------------------------

310     TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT  369
421     TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT  480
395     TATCCCCTTCCCTGGGAGAAGGACACAGAGAAACTGGTAGCTTTCTTGTTTGGAATTACT  454
        ------------------------------------------------------------

370     TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG  429
481     TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG  540
541     TCTCACATGGCGGCAGATGTCAGCTGGCATAGTCTGGGCCTTGAACAAGGATTCCTTAGG  514
        ------------------------------------------------------------

430     ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT  489
541     ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT  600
515     ACCATGGGAGCTATTGATTTTCACGGCTCCTATTCAGAGGCTCATTCGGCTGGTGATTTT  574
        ------------------------------------------------------------
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8.2

```
490   GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT   549
601   GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT   660
575   GGAGGAGATGTGTTGAGCCAGTTTGAATTTAATTTTAATTACCTTGCACGACGCTGGTAT   634
      ------------------------------------------------------------

550   GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC   609
661   GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC   720
635   GTGCCAGTCAAAGATCTACTGGGAATTTATGAGAAACTGTATGGTCGAAAAGTCATCACC   694
      ------------------------------------------------------------

610   GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA   669
721   GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA   780
695   GAAAATGTAATCGTTGATTGTTCACATATCCAGTTCTTAGAAATGTATGGTGAGATGCTA   754
      ------------------------------------------------------------

670   GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC   729
781   GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC   840
755   GCTGTTTCCAAGTTATATCCCACTTACTCTACAAAGTCCCCGTTTTTGGTGGAACAATTC   814
      ------------------------------------------------------------

730   CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT   789
841   CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT   900
815   CAAGAGTATTTTCTTGGAGGACTGGATGATATGGCATTTTGGTCCACTAATATTTACCAT   874
      --------------------------------------------------GGGCTGTAAC   10

790   CTAACAATCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG   849
901   CTAACAAGCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG   960
875   CTAACAAGCTTCATGTTGGAGAATGGGACCAGTGACTGCAACCTGCCTGAGAACCCTCTG   934
11    TCTGCCATCCCTCAGCATAATTTGGGGGTATGATTTCACTATCCTAATTGCCTGTCCTAA   70

850   TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT   909
961   TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT   1020
935   TTCATTGCATGTGGCGGCCAGCAAAACCACACCCAGGGCTCAAAAATGCAGAAAAATGAT   994
71    GTGATCTTACTTGCTGATAGGACCTAATGTTTTATTTTATTGTTTAGCACTTCTAAAAAC   130

910   TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT   969
1021  TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT   1080
995   TTTCACAGAAATTTGACTACATCCCTAACTGAAAGTGTTGACAGGAATATAAACTATACT   1054
131   TCATTTCCTTTACACAAGTCCAATACTTTGGACAGGAAACAGTAGCTTTGTTGATTATGC   180

970   GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC   1029
1081  GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC   1140
1055  GAAAGAGGAGTGTTCTTTAGTGTAAATTCCTGGACCCCGGATTCCATGTCCTTTATCTAC   1114
181   TACGTGTCTTTACTGTCTATAATGATTCTTTTATTTCAGGATTCCATGTCCTTTATCTAC   240

1030  AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG   1089
1141  AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG   1200
1115  AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG   1174
241   AAGGCTTTGGAAAGGAACATAAGGACAATGTTCATAGGTGGCTCTCAGTTGTCACAAAAG   300

1090  CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG   1149
1201  CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG   1260
1175  CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG   1234
301   CACGTCTCCAGCCCCTTAGCATCTTACTTCTTGTCATTTCCTTATGCGAGGCTTGGCTGG   360
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8.3

15/18

1150 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1209
1261 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1320
1235 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 1294
361 GCAATGACCTCAGCTGACCTCAACCAGGATGGGCACGGTGACCTCGTGGTGGGCGCACCA 420

1210 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1269
1321 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1380
1295 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 1354
421 GGCTACAGCCGCCCCGGCCACATCCACATCGGGCGCGTGTACCTCATCTACGGCAATGAC 480

1270 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1329
1381 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1440
1355 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 1414
481 CTGGGCCTGCCACCTGTTGACCTGGACCTGGACAAGGAGGCCCACAGGATCCTTGAAGGC 540

1330 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1389
1441 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1500
1415 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 1474
541 TTCCAGCCCTCAGGTCGGTTTGGCTCGGCCTTGGCTGTGTTGGACTTTAACGTGGACGGC 600

1390 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1449
1501 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1560
1475 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 1534
601 GTGCCTGACCTGGCCGTGGGAGCTCCCTCGGTGGGCTCCGAGCAGCTCACCTACAAAGGT 660

1450 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1509
1561 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1620
1535 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 1594
661 GCCGTGTATGTCTACTTTGGTTCCAAACAAGGAGGAATGTCTTCTTCCCCTAACATCACC 720

1510 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1569
1621 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1680
1595 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 1654
721 ATTTCTTGCCAGGACATCTACTGTAACTTGGGCTGGACTCTCTTGGCTGCAGATGTGAAT 780

1570 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1629
1681 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1740
1655 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 1714
781 GGAGACAGTGAACCCGATCTGGTCATCGGCTCCCCTTTTGCACCAGGTGGAGGGAAGCAG 840

1630 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC 1689
1741 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC 1800
1715 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC 1774
841 AAGGGAATTGTGGCTGCGTTTTATTCTGGCCCCAGCCTGAGCGACAAAGAAAAACTGAAC 900

1690 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 1749
1801 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 1860
1775 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 1834
901 GTGGAGGCAGCCAACTGGACGGTGAGAGGCGAGGAAGACTTCTCCTGGTTTGGATATTCC 960

1750 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGAGCCCGACCTGGAAG 1809
1861 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGAGCCCGACCTGGAAG 1920
1835 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGAGCCCGACCTGGAAG 1894
961 CTTCACGGTGTCACTGTGGACAACAGAACCTTGCTGTTGGTTGGAGCCCGACCTGGAAG 1020

8.4

```
1810 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1869
1921 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1980
1895 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1954
1021 AATGCCAGCAGGCTGGGCCATTTGTTACACATCCGAGATGAGAAAAAGAGCCTTGGGAGG 1080

1870 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 1929
1981 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 2040
1955 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 2014
1081 GTGTATGGCTACTTCCCACCAAACGGCCAAAGCTGGTTTACCATTTCTGGAGACAAGGCA 1140

1930 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 1989
2041 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 2100
2015 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 2074
1141 ATGGGGAAACTGGGTACTTCCCTTTCCAGTGGCCACGTACTGATGAATGGGACTCTGAAA 1200

1990 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 2049
2101 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 2160
2075 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 2134
1201 CAAGTGCTGCTGGTTGGAGCCCCTACGTACGATGACGTGTCTAAGGTGGCATTCCTGACC 1260

2050 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 2109
2161 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 2220
2135 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 2194
1261 GTGACCCTACACCAAGGCGGAGCCACTCGCATGTACGCACTCATATCTGACGCGCAGCCT 1320

2110 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 2169
2221 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 2280
2195 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 2254
1321 CTGCTGCTCAGCACCTTCAGCGGAGACCGCCGCTTCTCCCGATTTGGTGGCGTTCTGCAC 1380

2170 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 2229
2281 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 2340
2255 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 2314
1381 TTGAGTGACCTGGATGATGATGGCTTAGATGAAATCATCATGGCAGCCCCCCTGAGGATA 1440

2230 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 2289
2341 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 2400
2315 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 2374
1441 GCAGATGTAACCTCTGGACTGATTGGGGGAGAAGACGGCCGAGTATATGTATATAATGGC 1500

2290 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 2349
2401 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 2460
2375 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 2434
1501 AAAGAGACCACCCTTGGTGACATGACTGGCAAATGCAAATCATGGATAACTCCATGTCCA 1560

2350 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 2409
2461 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 2520
2435 GAAGAAAAGGTAAGTGAAAAAAAAAAAAAAAAAAAAAA---------------------- 2472
1561 GAAGAAAAGGCCCAATATGTATTGATTTCTCCTGAAGCCAGCTCAAGGTTTGGGAGCTCC 1620

2410 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 2469
2521 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 2580
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1621 CTCATCACCGTGAGGTCCAAGGCAAAGAACCAAGTCGTCATTGCTGCTGGAAGGAGTTCT 1680
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3130 AAACAACACACACTGGGGCCTATGAGAGGGCGGAAGGTGGGAGGAGGGAGAGATCAGGAA 3189
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3190 AAATAACTAATGGATACTTAGGGTGATGAAATAATCTGTGTAACAAACCCCCATGACACA 3249
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3250 CCTTTATGTATGTAACAAACCAGCACTTCCTGCGCATGTACCCCTGAACTTAAAAGTTAA 3309
     ------------------------------------------------------------
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3310 AAAAAAGTTGAACTTAAAAATAACAGATTGGCCCATGCCAATCAAAGTATAATAGAAAGC 3369
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     ------------------------------------------------------------

3370 ATAGTATAC 3378
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